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A subterranean species of *Exocelina* diving beetle from the Malay Peninsula filling a 4,000 km distribution gap between Melanesia and southern China

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Abstract

We describe a new subterranean species of the genus *Exocelina* Broun, 1886 (Coleoptera: Dytiscidae) from the Malay Peninsula. Almost all of the 196 species of that genus are epigean and distributed mainly in New Guinea, Australia, Oceania and New Caledonia. One epigean species is, however, known from China. The discovery of a species on the Malay Peninsula fills that distribution gap to some degree.

Keywords

Beetles, blind subterranean species, disjunct distribution, new species

Introduction

Here we report the discovery of a new subterranean diving beetle from the Malay Peninsula. This species was placed in the Dytiscidae, subfamily Copelatinae based on morphological characters using the key of Miller and Bergsten (2016). It was then unambiguously assigned to the genus *Exocelina* Broun, 1886 in a phylogenetic analysis using molecular systematic data of Toussaint et al. (2014, 2015, 2020 in preparation).

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The 196 described species of *Exocelina* are mostly from New Guinea (141 species, see e.g. Balke 1998; Shaverdo et al. 2018, 2019; Shaverdo and Balke 2019), followed by New Caledonia (37 species) and Australia (16 species, two of them subterranean), with single species each in Hawaii and Vanuatu (Balke et al. 2007; Nilsson and Hájek 2019). All of these localities lie east of the Lydekkers line. A single species was discovered in Shizong, Yunnan, China (Balke and Bergsten 2003), leaving a gap of around 4,000 km in the distributional range of *Exocelina*, essentially the entire Indonesian Archipelago and mainland Southeast Asia. The present finding partly fills this gap and suggests that more discoveries are to be expected, for example from the little sampled mountain regions of Vietnam and Laos. A synopsis of the subterranean diving beetles of the World was provided by Miller and Bergsten (2016), who provide an identification key as well as habitus photographs.

Material and methods

Specimens were studied with a Leica M205C stereo microscope at 10–160x. Images were taken with a Canon EOS 5DS camera fitted with a Mitutoyo 10x ELWD Plan Apo objective attached to a Carl Zeiss Jena Sonnar 3.5 / 135 MC as focus lens. Illumination was with two to four LED segments SN-1 from Stonemaster (https://www.stonemaster-onlineshop.de). Image stacks were generated using the Stackmaster macro rail (Stonemaster), and images were then assembled with the computer software Helicon Focus 4.77TM.

Drawings were produced with a camera lucida, first sketched with pencil on paper, then photographed and digitally inked using an iPad Pro and the Concepts as well as MediBang Paint APPs.

One paratype male of the new species (voucher number IBE-AN1160) was used for a non-destructive DNA extraction using a commercial kit (Qiagen DNeasy Tissue Kit). We successfully amplified six mitochondrial and nuclear genes in five sequencing reactions, two cytochrome c oxidase subunit I fragments (COI-5' -the "barcode"- and COI-3'), 5' end of rrnL RNA plus leucine tRNA transfer (tRNA-L1) plus 5' end of NADH dehydrogenase subunit I (NAD1), and one internal fragment of both small ribosomal unit (18S RNA) and Histone 3 (H3) (see Villastrigo et al. 2018, for details of the primers and sequencing conditions). These are fragments routinely used for Dytiscidae systematics. Sequences were edited using Geneious v10.1 (Kearse et al. 2012). Here, we combined the newly obtained sequences of COI-3', 18S and H3 (ENA database with accession numbers LR759936 H3, LR759937 18S, LR759938 3'COI, LR760127 5'COI) with the data of Toussaint et al. (2014, 2015 as well as 2020 in preparation). Other markers used by the latter authors (such as Carbomoylphosphate synthase (CAD) and Alpha-Spectrin (Asp)) could not be amplified here.

The combined dataset was analysed with a fast maximum likelihood search as implemented in IQ-TREE v1.6 (Nguyen et al. 2015), with a partition by gene fragment and the best evolutionary model as selected by Modelfinder (Kalyaanamoorthy et al. 2017) using the AIC (Akaike Information Criterion). We assessed topological stability

with 1000 ultrafast bootstraps and tested tree branches by SH-like aLRT with 1000 replicates (Nguyen et al. 2015).

Repositories

IBE Institute of Evolutionary Biology, Barcelona, Spain
KSc Kazuki Sugaya collection, Zama, Japan
NMW Naturhistorisches Museum Wien, Austria
ZSM Zoologische Staatssammlung München, München, Germany

Taxonomy

Family Dytiscidae Leach, 1815
Genus *Exocelina* Broun, 1886

***Exocelina sugayai* sp. nov.**
http://zoobank.org/D7A59208-6691-4E3E-8899-9942AC745D4A

Type locality. Malaysia, Pahang, Cameron Highlands, Tanah Rata, 4.474705, 101.384043.

Material examined. ***Holotype male* (ZSM):** Malaysia, Pahang, Cameron Highlands, Tanah Rata, Mount Berembun, 4.474705, 101.384043, 1,500m, 27.–29.ii.2012, K. Sugaya leg.

***Paratypes*:** 4 males (1 used for DNA extraction and sequencing, voucher No. IBE-AN1160) and 2 females, same label data as holotype (IBE, KSc, NMW, ZSM).

Description of holotype. ***Size and shape***: Smallest *Exocelina* known (length of holotype including head 2.7 mm, length without head 2.4 mm, greatest width 1.0 mm). Abdomen comparably parallel sided; pronotum also comparably parallel sided, slightly constricted before base, hind angles produced backwards (Fig. 1A).

Coloration. Testaceous and slightly translucent (Figs 1A, B, 2A–F).

Surface sculpture. Head and pronotum with distinct microreticulation formed by small regular cells and fine moderately dense punctation. Elytra with distinct microreticulation formed by small regular cells and dense, coarse, setiferous punctation (Fig. 1A, D). Ventral side with distinct microreticulation formed by small regular cells, including distinct microreticulation on metacoxal processes (Figs 3A, 4A–C).

Structures. Eyes fully reduced, with only small black scars remaining on surface of head (Figs 1A, B, 2A, B). Male antennomeres strongly modified: 2 and 3 moniliform, 4 slightly broadened in dorsal view, 5–11 strongly expanded, 11 flat and blade like (Fig. 1A). Fore tarsus dilated, fore angle of tarsomere 4 ventrally produced (Fig. 1C) and with two thicker setae (but no hook as in other *Exocelina*), on tarsomere 5 ventrally without obvious setation; pro and mesotarsomeres 1–3 with 4 rows of stalked suction discs (2 per row). Pronotum with faint lateral bead not reaching an-

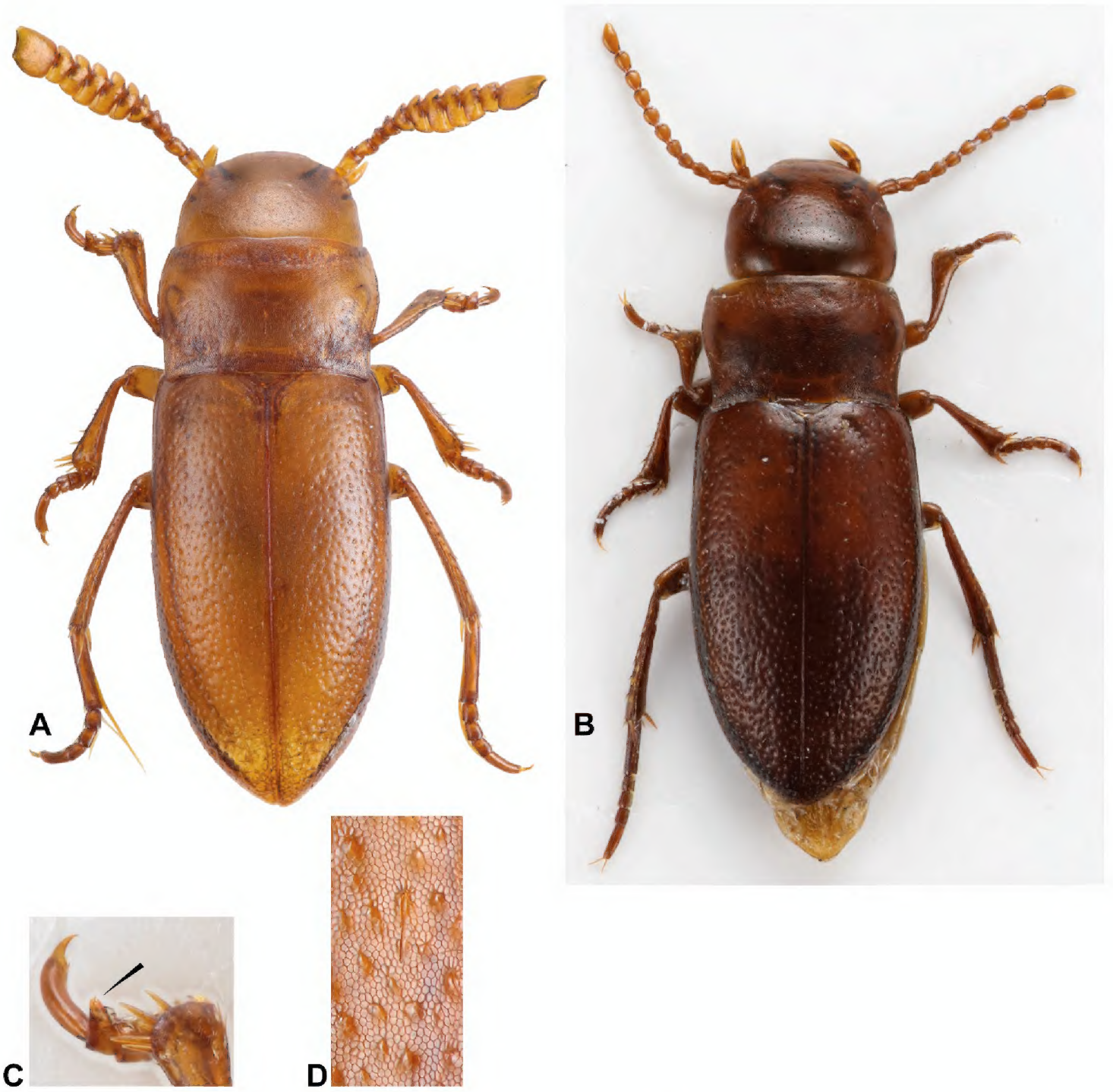

Figure 1. *Exocelina sugayai* sp. nov. **A** habitus dorsal of male **B** same of female **C** foretarsus of male, arrow pointing at expanded anterior ventral angle of tarsomere IV **D** surface sculpture on male elytral disc, cropped from A. Length of left beetle: 2.7 mm.

terior nor posterior corners (Fig. 2B, D, F). Prosternal process short, lanceolate, deflexed, gently rounded ventrally (Figs 3A, 4A); metaventrite broadly triangular, its lateral "wings" very narrow (Fig. 4B, C). Membranous wings strongly reduced, with only very short stubs visible at the wing base. Metacoxal "lines" broadly diverging, fainting well before hind margin of metaventrite (Figs 3A, 4B). Metacoxal processes small, more elongate oval, with wide gap in middle (to possibly enable higher mobility of hindlegs) (Figs 3A, 4B). Last ventrite apically rounded. Median lobe of aedeagus simply curved in lateral view, parameres of simple, Copelatinae-type triangular shape (Fig. 5A, B).

Female. Antennomeres filiform to slightly moniliform (Fig. 1B). Pro and mesotarsomeres 1–3 not bearing stalked suction discs and protarsomere 4 not modified.

Figure 2. *Exocelina sugayai* sp. nov. male **A** eye in lateral view **B** detail of head and pronotum **C** surface sculpture on base of head and anterior margin of pronotum **D** detail of posterior angle of pronotum **E** detail of surface sculpture on base of elytron **F** detail of lateral view of elytral and pronotal base and head.

Figure 3. Ventral view of **A** *Exocelina sugayai* sp. nov. male and **B** *Exocelina abdita*.

Variation. Length of beetle including head 2.4–2.8 mm. Two paratypes are darker orange (see Fig. 1B). According to the collector, this is due to subsequent darkening in alcohol storage.

Etymology. Named after Kazuki Sugaya, the discoverer of this species.

Differential diagnosis. This species differs from all other Dytiscidae by: Copelatinae with reduced eyes; beetle length < 3 mm; body with well visible microreticulation; prosternal process short and deflexed; metacoxal processes small, more elongate oval (in other Copelatinae, including the groundwater species *Exocelina abdita* Balke et al. 2004, this structure is more rounded, and the metacoxal "lines" can be more parallel sided, Figs 3B, 4D); male with strongly modified antennomeres.

Figure 4. *Exocelina sugayai* sp. nov. male, ventral side **A** prosternal process and mesocoxal area **B** metacoxa and metacoxal processes **C** metaventrite and metaxoca **D** *Exocelina abdita*, metacoxa and metacoxal processes. Lines in **B** and **D** inserted to highlight outline of metacoxal processes.

Habitat. Collected from two helocrenes on a slope in forested area. The beetles were observed creeping around and were not swimming when observed (K. Sugaya personal communication 2019) (Fig. 6A, B).

Phylogenetic affinities. The best evolutionary model fitting the data according to Modelfinder was a GTR+F for all partitions. *Exocelina sugayai* sp. nov. was recovered deeply subordinated within *Exocelina*, as the sister of the Chinese *E. shizong* Balke &

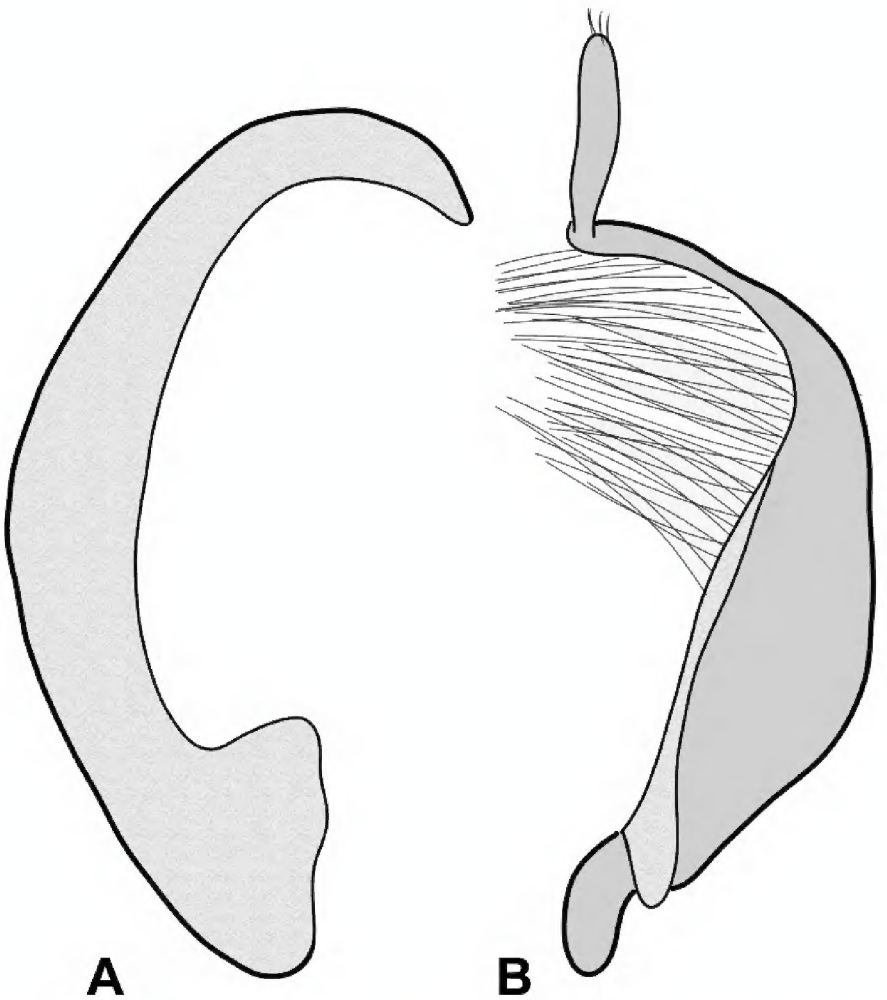

Figure 5. *Exocelina sugayai* sp. nov. male genital, **A** median lobe of aedeagus in lateral view **B** paramere lateral inner view.

Bergsten, 2003 and the New Caledonian *E. nehoue* Balke et al., 2014. These three species are part of a clade ("C4" in Toussaint et al. 2015) otherwise containing *E. parvula* (Boisduval, 1835) from Hawaii as well as a clade of New Caledonian and one Vanuatu species (Fig. 7). The other two subterranean species of *Exocelina* are *E. abdita* Balke et al., 2004 and *E. rasjadi* Watts & Humphreys, 2009 from Australia. The former was included in our phylogenetic analysis and placed in a different clade than *Exocelina sugayai* sp. nov. (Fig. 7, included subterranean species in red). Data for *E. rasjadi* were not available.

Discussion

Most species of *Exocelina* inhabit stream associated (lotic) habitats, specifically areas of stagnant water at the edge of streams and creeks, the interstitial and tiniest of water holes on riverbanks, as well as small puddles in intermittent creeks including the source area that might only have occasional water flow after rainfalls (see habitat photos in Shaverdo et al. 2012). This is the likely ancestral habitat type in *Exocelina*, with four subsequent shifts to lentic habitats (and only a few species in the lentic clades) (Toussaint et al. 2015). Most species have limited geographic ranges; in one widespread epigean species population genomic studies revealed strong geographic structure even in populations as close to each other as 40 km straight line (Lam et al. 2018).

Figure 6. Habitat of *Exocelina sugayai* sp. nov. **A** overview **B** detailed, with a beetle crawling about in the center of the image.

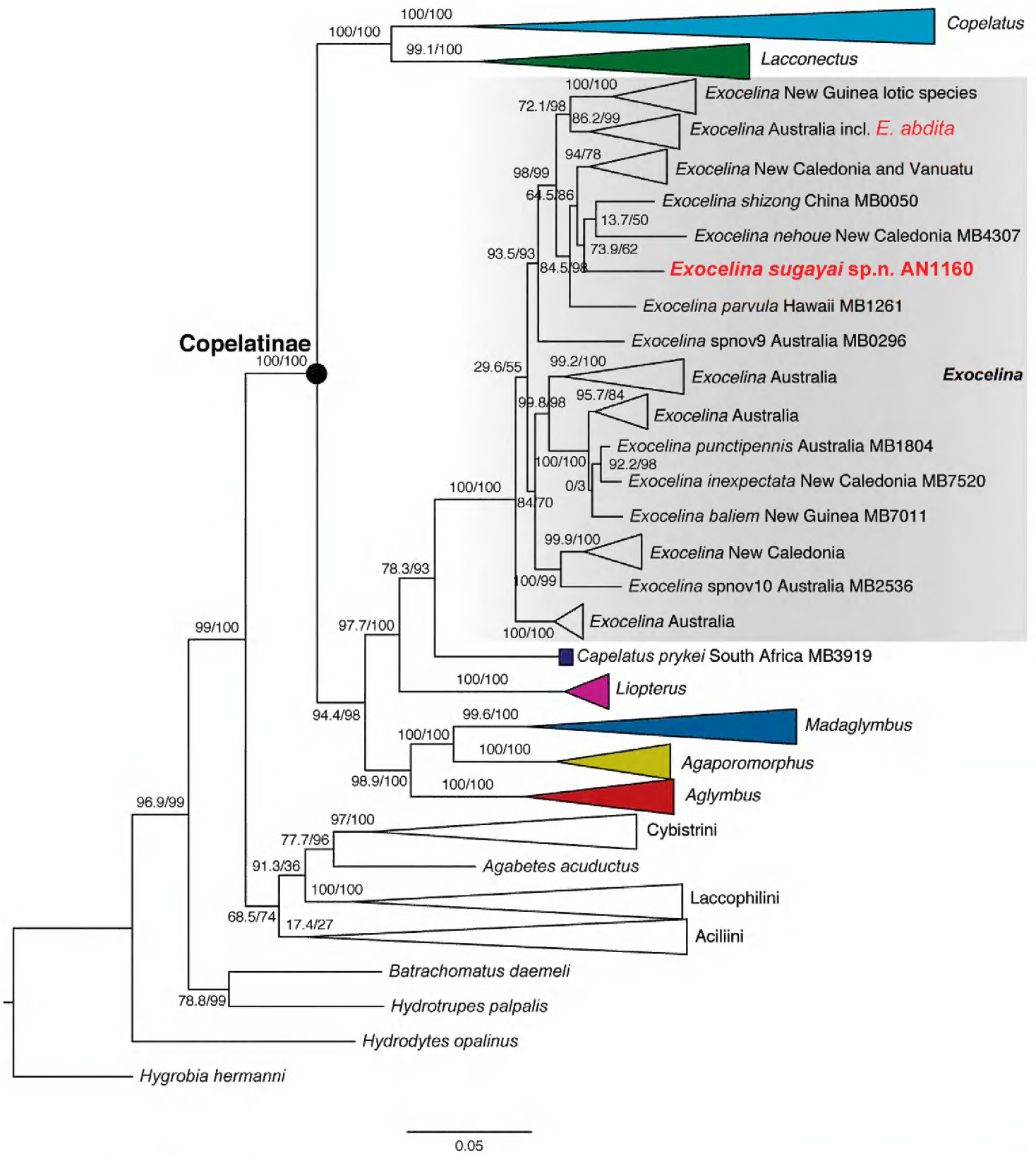

Figure 7. Simplified phylogenetic tree obtained with IQ-TREE using the DNA sequence dataset of Toussaint et al. (2014, 2015 as well as 2020 in preparation) plus the newly obtained sequences of *Exocelina sugayai* sp. nov. Non-relevant clades are collapsed to genus or other major clades. Numbers in nodes, ultrafast bootstrap / SH-like aLRT support.

The lotic beetles often hide in the gravel when disturbed, and observations of M. Balke in New Guinea suggest that the interstitial of riverbanks is often utilized by these beetles, possibly to avoid downstream drift. The beetles seem to avoid habitat with fine, dense substrates, which we suggest make it hard to hide as such substrate clogs the space between stones and pebbles (see also Balke 2001).

This lifestyle could be interpreted as a preadaptation for interstitial or stygobitic life. In fact, some Australian species seem to mainly inhabit the interstitial, and have been suggested to provide a scenario for the transition from epigean to stygobitic life (Watts et al. 2016). To date, two species have been described from groundwater habitats in Australia. They exhibit a strongly modified morphology typical of stygobitic species, such as wing and eye reduction and depigmentation (Balke et al. 2004; Watts and Humphreys 2009, see also Watts et al. 2016). The discovery of the new species described here suggests that many more such stygobitic *Exocelina* could be found in the future. Our phylogenetic analysis also suggests that the evolution of subterranean *Exocelina* occurred at least two times independently (Fig. 7). In Copelatinae, one species of the genus *Copelatus* Erichson, 1832 from Brazil has been described from the subterranean habitat (Caetano et al. 2013).

Biogeographically, the occurrence of Southeast Asian and a Chinese species of *Exocelina* remains enigmatic. The origin of the clade containing these species was estimated as at least 10 million years ago ("C4" Toussaint et al. 2015). Based on the information currently available, we can not state with confidence whether the Asian species are "relics" of a previously diverse and widespread *Exocelina* fauna, or the result of rare dispersal events without apparent subsequent diversification.

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